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Bernadette B. Fahey — April 23, 2002
Bernadette B. Fahey — Date

Att. Docket No REG 900A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application Of	:	Nicholas W. Gale, et al.
USSN	:	10/055,842
Filed	:	January 23, 2002
For	:	METHODS OF IMAGING AND TARGETING VASCULATURE
		April 23, 2002

Commissioner of Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

AMENDMENT AND RESPONSE TO FEBRUARY 25, 2002 NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

Sir:

A response to the February 25, 2002 Notice to File Missing Parts of Nonprovisional Application ("Notice') is due on April 25, 2002, and, therefore, this response is being timely filed.

In response to the February 25, 2002, Notice, Applicants enclose the following: Exhibit A: a copy of the Notice to File Missing Parts; Exhibit B: executed Declaration and Power of Attorney, Exhibit 3: copies of Marked-Up Versions of pages 11, and 19. The Commissioner is hereby authorized to charge the required fee of $130.00 to Deposit Account 18-0650.

Prior to examination of the application on its merits, please amend the specification as follows:

IN THE SPECIFICATION:

Please replace the paragraph starting on page 11, line 6, with the following:

Figure 1A-1G. ephrin-B2 is highly expressed at sites of secondary angiogenesis in the embryo, as well as at sites of normal and pathological angiogenesis in the adult. **Figure 1A-1G** are from ephrin-B2/LacZ mice. **Figure 1A-1B:** ephrin-B2/LacZ is strongly expressed during physiological maturing ovarian follicles (**Figure 1A**) (arrows indicate capillaries) and in the neovasculature (arrowheads) of the corpus luteum after ovulation (**Figure 1B**). **Figure 1C-1D:** ephrin-B2/LacZ is strongly expressed in the neovasculature of a subcutaneously grown tumor (Lewis Lung Carcinoma). At high magnification LacZ staining is absent in veins (indicated by closed arrowheads), but is found in longitudinally oriented endothelial cells (open arrowheads in **Figure 1D**), also seen in a higher power view (inset). **Figure 1E-1G:** ephrin-B2 expression in a subset of tumor endothelial cells. (**Figure 1E**) Beta-galactosidase immunostaining marks a proportion of the endothelial cells labeled by PECAM (**Figure 1F**). Corresponding regions between **Figure 1E** and **Figure 1F** indicated by white arrowheads). **Figure 1G:** In a section of tumor dual-labeled for beta-galactosidase and smooth muscle actin (SMA), ephrin-B2 activity is found in endothelial cells (green arrowheads), but not in pericytes (red arrowheads).

Please replace the paragraph starting on page 19, line 5, with the following:

To examine the expression of ephrin-B2 during pathologic angiogenesis, subcutaneous tumors were examined. In a dramatic example of one such tumor that had invaded the underlying muscle, it can be seen that certain vessels growing into the tumor from the surrounding muscle express high levels of ephrin-B2 (Figure 1C). Consistent with the notion that ephrin-B2 marks arterial and not venous vessels, these tumor vessels appear to arise from previously existing ephrin-B2-expressing arterioles within the muscle (Figure 1C). These data suggest that tumor vessels, which were previously assumed to consist of homogenous capillaries based on their small size and paucity of smooth muscle investiture (e.g. Folkman, 1971), may be divided into microvessels with either arterial or venous identity. Further consistent with this notion, it is clear that only a subset of the new tumor vessels are ephrin-B2 positive (Figure 1D-F). Finally, ephrin-B2 was expressed by the endothelium of tumor vessels (Figure 1D and 1G); smooth muscle cells associated with tumor vessels clearly did not express ephrin-B2.

REMARKS

This amendment is being made merely to conform the specification to the drawings as filed. No new matter is added by this amendment and

Applicants respectfully request entry of the amendment into the specification.

No additional fee is deemed necessary. However, if any additional fee is required, the Commissioner is hereby authorized to charge any such additional fee to Deposit Account No. 18-0650.

Respectfully submitted,

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capable of dissolving a blood clot in a blood vessel, or is capable of reducing atherosclerotic plaques.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1A-~~1I~~1G. ephrin-B2 is highly expressed at sites of secondary angiogenesis in the embryo, as well as at sites of normal and pathological angiogenesis in the adult. **Figure 1A-~~1I~~1G** are from ephrin-B2/LacZ mice. **Figure 1A-1B:** ephrin-B2/LacZ is strongly expressed during physiological maturing ovarian follicles (**Figure 1A**) (arrows indicate capillaries) and in the neovasculature (arrowheads) of the corpus luteum after ovulation (**Figure 1B**).

Figure 1C-1D: ephrin-B2/LacZ is strongly expressed in the neovasculature of a subcutaneously grown tumor (Lewis Lung Carcinoma). At high magnification LacZ staining is absent in veins (indcated by closed arrowheads), but is found in longituninally oriented endothelial cells (open arrowheads in **Figure 1D**), also seen in a higher power view (inset).

Figure 1E-1G: ephrin-B2 expression in a subset of tumor endothelial cells. (**Figure 1E**) Beta-galactosidase immunostaining marks a proportion of the endothelial cells labeled by PECAM (**Figure 1F**). Corresponding regions between **Figure 1E** and **Figure 1F** indicated by white arrowheads).

Figure 1G: In a section of tumor dual-labeled for beta-galactosidase and smooth muscle actin (SMA), ephrin-B2 activity is found in endothelial cells (green arrowheads), but not in pericytes (red arrowheads).

found that ephrin-B2 expression specifically marks arterial as opposed to venous vessels in the adult. Furthermore, ephrinB2 expression is upregulated in situations of new blood vessel formation (angiogenesis).

To examine the expression of ephrin-B2 during pathologic angiogenesis, subcutaneous tumors were examined. In a dramatic example of one such tumor that had invaded the underlying muscle, it can be seen that certain vessels growing into the tumor from the surrounding muscle express high levels of ephrin-B2 (Figure ~~1E (4E)~~ 1C). Consistent with the notion that ephrin-B2 marks arterial and not venous vessels, these tumor vessels appear to arise from previously existing ephrin-B2-expressing arterioles within the muscle (Figure ~~1E (4E)~~ 1C). These data suggest that tumor vessels, which were previously assumed to consist of homogenous capillaries based on their small size and paucity of smooth muscle investiture (e.g. Folkman, 1971), may be divided into microvessels with either arterial or venous identity. Further consistent with this notion, it is clear that only a subset of the new tumor vessels are ephrin-B2 positive (Figure ~~1F-1H (4F-H)~~ 1D-F). Finally, ephrin-B2 was expressed by the endothelium of tumor vessels (Figure ~~1F and 1I (4F & I)~~ 1D and 1G); smooth muscle cells associated with tumor vessels clearly did not express ephrin-B2.

In adult settings of physiologic and pathologic angiogenesis, such as during remodeling of the female reproductive system or in tumors, ephrin-B2 seems to recapitulate its earliest patterns of embryonic expression. That is, ephrin-B2 is highly expressed by the endothelium of some angiogenic vessels and their sprouts, and is largely lacking from the few smooth muscle cells that are associated with new vessels. The finding that angiogenic sprouts at sites of adult neovascularization have arterial identity challenges prevailing views that these sprouts largely derive from